

Research article

A study of inflammatory markers and their correlation with PASI score in psoriasis – A case control studyNeela B. Mannangi¹, Basavaraj Devaranavadi², Shubha Jayaram³, Balachandra S. Ankad⁴, Shankarprasad D.S.¹¹Department of Biochemistry, ⁴Department of Dermatology, SNMC & HSK, RC, Bagalkot, Karnataka, India²Department of Biochemistry, BLDE Deemed to be University, Vijayapur, Karnataka, India³Department of Biochemistry, Mysore Medical College & Research Institute, Mysore, Karnataka, India

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ABSTRACT

Introduction and Aim: Psoriasis is one of the salient dermatological disorder and a chronic recurrent cutaneous disease. Research shows that immunological cells and cytokines play a principal causative role for skin lesions and comorbid systemic effects in these patients. Like other autoimmune diseases, psoriasis is also a multifactorial disorder which is triggered either by injury, trauma, infections, medications, and psychological stress. Among the cytokines, hs-CRP acts as both inflammatory and cardiovascular marker. It is markedly increased in psoriasis patients, thereby leading to subsequent co-morbidities in these patients. The aim of present study was to estimate inflammatory markers viz-TNF- α , IL-2, IFN- γ and hs-CRP in psoriasis patients and controls and correlate these inflammatory markers with PASI score in psoriasis patients. Further to correlate hs-CRP with TNF- α , IL-2, IFN- γ in these patients.

Materials and Methods: The study is conducted at a tertiary level hospital and is designed as a Case Control study design conducted from January 2019 to January 2021. 110 subjects having diagnosed as psoriasis were included as cases and 110 controls. Inflammatory markers were estimated by ELISA method. Statistical analysis was done.

Results: The study showed that inflammatory markers in cases with psoriasis were significantly elevated when compared with controls. The inflammatory markers were proportionately altered from mild to severe in psoriasis patients but were not statistically significant. The hs-CRP showed negative significant correlation with IFN- γ .

Conclusion: The present study concludes that the inflammatory markers are significantly increased in psoriasis patients, and this is correlated with PASI score. These simple biomarkers are of utmost importance in the clinical scenario for better treatment and prognosis and thereby reducing comorbidities in these patients.

Keywords: TNF α ; IFN- γ ; IL-2; hs-CRP; psoriasis.

INTRODUCTION

Psoriasis is the commonest and chronic recurrent cutaneous disorder, which is distinguished by the excessive proliferation and extremely increased rate of epidermal turnover along with infiltration of activated mononuclear cells in the underlying dermis (1). The prevalence rate of psoriasis is 2–3% which signifies the epidemiological burden. The disease is predominant in the polar regions, however, tropical/ subtropical countries like India, also show significant burden of Psoriasis. India being a diverse country, there is a regional variation in the prevalence of Psoriasis with variable environmental and genetic factors (2). Based on previous studies it is inferred that in India there is a natural regional variation in the prevalence of psoriasis from 0.44 to 2.8%, and males are affected twice more than females. At the time of presentation, most of the patients are in their thirties or forties (3).

Psoriasis exhibits altered immune response with cutaneous involvement which induces a chronic inflammation of the skin. The immunological cells and their cytokines play an important causative role for skin lesions and comorbid systemic effects (4). The cutaneous tissue suffers a major influence on the disease outcome. In this cutaneous disease T cells and cytokines play an important role for the pathogenesis of the disease. Several studies have confirmed that immune system is impaired in psoriasis. Some studies have also mentioned that psoriasis is an immune mediated disorder with abnormal proliferation of keratinocytes which is further aggravated and mediated by T-lymphocytes. Psoriasis is being associated with an over expression of proinflammatory cytokines released by T helper1(Th1) cells and relative presence of pro-inflammatory cells. Their cytokines create a damaging environment leading to the development and further aggravation of psoriatic lesions (5).

In psoriasis the pattern is significantly complex. Th-1 cells are known to produce tumor necrosis factor-alpha (TNF- α), interleukin 2 (IL-2) and interferon-gamma (IFN- γ) under the effect of IL-12. In the same way, IL-1 β and IL-6 are responsible for Th17 cell differentiation, which secretes IL-6, IL-17, IL-21 and IL-22. The recruitment and activation of Th1 and Th17 lymphocytes, thus drive the pathogenesis of psoriasis. On the other hand, neutrophils, antigen presentation cells (APCs), macrophages and keratinocytes contribute for the synthesis and secretion of cytokines (6)

TNF- α is a cytokine secreted by T lymphocyte, keratinocytes, and dermal macrophages, CD11+ dendritic cells and mastocytes. TNF- α effects the synthesis of IL-6 and ICAM-1 expression, which in turn leads to hepatic stimulation with increased production of acute phase reactants mainly C-reactive protein (CRP) and fibrinogen (7). In Psoriasis one of the principal events in the pathogenesis of inflammatory outbreak is the secretion of IFN- γ from plasmacytoid dendritic cells (DCs). The Th-17-derived cytokines include IL-17A, IL-17F, IL-6, TNF- α , and IL-22. The hallmark of IL-22 activity is abnormal differentiation of keratinocytes along with increased hyperplasia resulting in plaque formation (8).

Psoriasis is shown to be characterized by increased levels of hs-CRP with increase in subclinical atherosclerosis (9). C-reactive protein is produced from hepatocyte within hours after being stimulated from inflammation, infection, tissue damage, and decreases rapidly when the stimulating factor has been eliminated or responded to treatment. This marker is over expressed in psoriasis (10).

Like other autoimmune diseases, psoriasis is also a multifactorial disorder which is triggered either by injury, trauma, infections, medications, and psychological stress (3). The etio-pathogenesis of psoriasis is less well understood. Nevertheless it has been shown that genetic and epigenetic factors play a strong role in this disease. In this cutaneous disease T-cells and cytokines overtly contribute to the pathogenesis of the disease (1).

The diagnosis of psoriasis done by the dermatologists is made by the clinical findings and Psoriasis Area and Severity Index (PASI) Score which is a tool for assessing the disease severity in these patients. This PASI score is assessed by the affected area and lesional characteristics. In these patients, the PASI score is directly proportional to the severity of the disease. A score of more than 12 is considered severe whereas a score of ≤ 12 is considered mild to moderate (11). It is very important to monitor and control inflammation to control the evolution of the disease and its comorbidities. It has been proved that IL-6 induces Type-2 DM (Diabetes mellitus) and significant cardiovascular adverse effects and that

TNF- α and hs-CRP could be involved in causing atherosclerosis (12).

Among the cytokines, hs-CRP acts as both inflammatory and cardiovascular marker. It is markedly increased in psoriasis patients, there by leading to co-morbidities in these patients. Several studies have shown the importance of cytokines in the clinico-pathogenesis of dermatological disorders. With this complexity role of cytokines, the present study is focused on estimation of these inflammatory markers in psoriasis patients compared to controls. Further hs-CRP is correlated with other inflammatory markers to assess cardiovascular comorbidities in future. There is paucity of literature concerning inflammatory markers profile and their correlation with PASI score in South Indian population. Hence the present study is undertaken.

MATERIALS AND METHODS

It is a case control study conducted in a tertiary care hospital attached to a medical college from January 2019 to August 2020. Total sample size was 220 in which 110 were cases and 110 controls. Sample size was determined according to a study done by Sandhya *et al.*, (13) using Open Epi software Version 2.3.1 with confidence level: 95% and power of the study: 80%. Calculation results were 100 in each group.

Inclusion criteria

The newly diagnosed patients with Psoriasis were included for the study. The severity of the disease was assessed and graded by the PASI score with Mild <7, Moderate 7-12, Severe >12 (11).

Exclusion criteria

Patients with any chronic inflammatory disease, diabetes mellitus, renal disorders, IHD (Ischemic Heart Disease), hypothyroidism, hyperthyroidism, nephritic syndrome, obstructive liver disease, and other skin disorders were excluded from the study. All the patients receiving systemic drug therapy like beta blockers, thiazides, retinoids, cyclosporine and lipid lowering agents in the recent 6 months were excluded from the study.

Approval was obtained from the institutional ethics committee. After taking informed consent, detailed history and clinical examination was done.

Under aseptic precautions around 5ml of blood was drawn in plain vacutainers and subjected to centrifugation at 3000 rpm for 20 minutes to separate the serum. Separated serum was used for analysing different biochemical parameters such as TNF- α , IL-2, IFN- γ and hs-CRP by ELISA method as per kit instructions supplied by Diaclone Technologies Laboratory.

Statistical analysis was done using SPSS software. The number and percentage were used for categorical data and significant difference between two categorical variables was tested by the Chi-square (χ^2) test. Unpaired ‘t’ test was used to compare between two independent variables as a test for difference of mean. The ANOVA and ‘F’ test were used if there were more than two independent groups for testing of equality of variance.

RESULTS

The present study had 110 cases (psoriasis) and 110 controls. Table 1 shows distribution of age in cases according to PASI score and controls. The gender

distribution with respect to PASI score in cases and controls is depicted in Fig. 1. Males were more than females both in cases and controls. The Mean \pm SD for inflammatory markers both in cases and controls are shown in Table 2. The Inflammatory markers were significantly increased in cases compared to controls.

Table 3 shows the distribution of inflammatory markers according to PASI score in cases and controls. There was no significant correlation between PASI score and inflammatory markers, however there was an increase trend of inflammatory markers in PASI score is seen.

Table 1: Distribution of age according to PASI score in cases and controls

Parameters (Mean \pm SD)	PASI score (Cases)			Control	‘F’ value	‘p’ value
	Mild	Moderate	Severe			
Age (yrs)	38.8 \pm 8.5	39.9 \pm 12.7	41.4 \pm 11.5	39.4 \pm 11.1	0.571	0.635

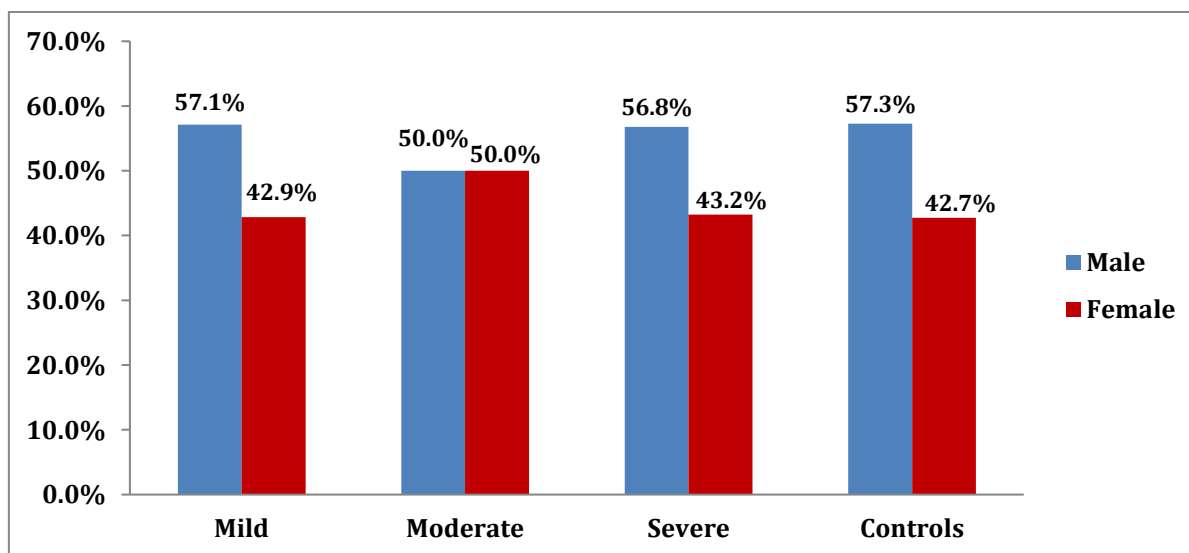


Fig. 1: Gender distribution with respect to PASI score in cases and controls

Table 2: Inflammatory markers in cases and controls (Mean \pm SD)

Inflammatory markers	Cases Mean \pm S.D	Controls Mean \pm S.D	‘t’	‘p’
TNF(pg/ml)	30.8 \pm 25.3	3.9 \pm 2.2	11.098	<0.001*
IFN- γ (pg/ml)	31.1 \pm 21.7	8.0 \pm 3.0	11.065	<0.001*
IL- 2(pg/ml)	25.7 \pm 10.3	11.7 \pm 4.1	13.144	<0.001*
hs-CRP	16.2 \pm 7.3	2.8 \pm 1.3	18.952	<0.001*

p value ‘*’ significant at 5% level of significance (p<0.05)

Table 3: Distribution of inflammatory markers in PASI score and controls

Inflammatory Markers (Mean ± SD)	Level of PASI Score			Control	F value	p value
	Mild	Moderate	Severe			
TNF(pg/ml)	22.4±10.3	27.4±24.9	33.3±27.1	3.9±2.2	43.468	<0.001*
γ - Interferon	23.5±12.4	35.6±19.9	31.3±23.4	8.1±3.1	43.189	<0.001*
IL- 2(pg/ml)	19.1±6	22.7±8.8	27.8±10.8	11.7±4.1	68.755	<0.001*
hs-CRP	16±6.6	15.8±6.4	16.3±7.7	2.8±1.3	118.79	<0.001*

p value ‘*’ significant at 5% level of significance (p<0.05)

Table 4: Correlation between hs-CRP and other inflammatory markers

Marker	TNF-α		γ-IFN		IL-2	
	‘r’	‘p’	‘r’	‘p’	‘r’	‘p’
hs-CRP	- 0.063	0.51	- 0.22	0.017*	0.051	0.60

p value ‘*’ significant at 5% level of significance (p<0.05)

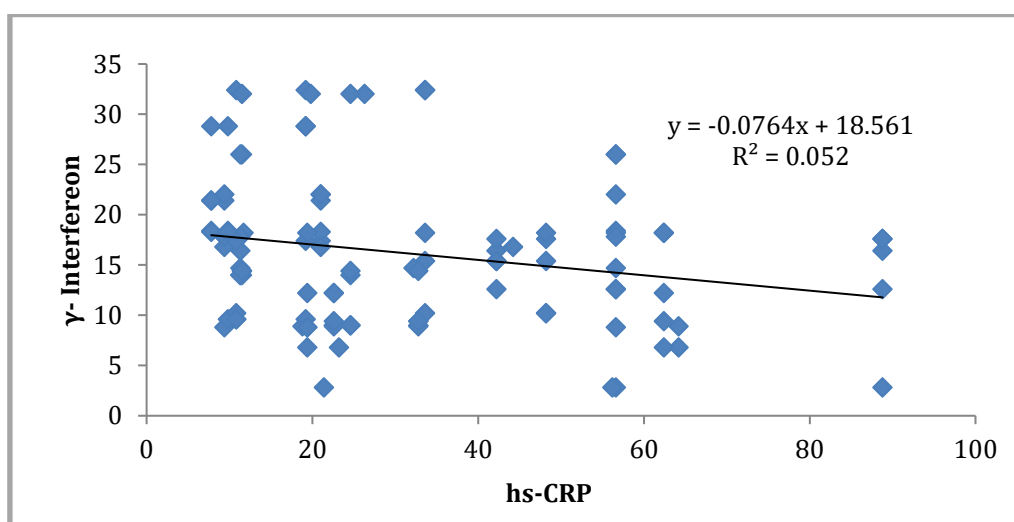


Fig. 2: Correlation between hs-CRP and γ-IFN

Hs-CRP being both inflammatory and cardiovascular marker was correlated with other inflammatory markers (TNF, γ – Interferon, IL- 2) as shown in Table 4. The hs-CRP showed negative correlation with TNF-α, γ-IFN and was statistically significant only with γ-IFN as shown in Fig.2 and positive correlation with IL-2 but was not statistically significant.

DISCUSSION

Psoriasis is one of the commonest and chronic recurrent cutaneous disease. In addition to cutaneous involvement it also involves nails and joints as in psoriatic arthritis (1). Based on previous studies it is inferred that in India there is a natural regional variation in the prevalence of Psoriasis from 0.44 to

2.8%, and males are affected twice more than females (3). It is manifested by the erythematous scaly plaques affecting all parts of the body, predominantly over elbows, knees, scalp, umbilical and perianal region. These lesions are the effects of inflammation, excessive proliferation and angiogenesis seen in psoriasis (3).

The inflammatory markers showed statistically significant increase in cases compared to controls. Cataldi *et al.*, also showed similar findings which is in accordance to our study (14). Macrophages and dendritic cells produce IL-12, which stimulates T effector cell differentiation into a pro-inflammatory Th1 response. Once activated, Th1 cells secrete cytokines which potentiates psoriasis pathophysiology.

Th1 stimulate release of cytokines such as IFN- γ , TNF- α and IL-2 (15). Th1 cells secrete and release IL-2, The major role of IL-2 is stimulation of the Th1 phenotype and thus producing IFN- γ , TNF- α and other pro-inflammatory cytokines, and activating Natural Killers (NK) cells (16). Some studies have shown no significant differences in the levels of IL-2 between psoriasis patients and healthy controls, however our study showed significant increase in IL-2 levels in psoriasis patients compared to controls. According to authors, the genetic differences in study populations could be the cause for discordant results (17). Studies have shown decreased serum IFN- γ levels in psoriasis patients which was not in accordance with the present study. According to authors IFN- γ signaling is multifaceted and complex, and the pathogenesis of which is not completely known. Recent studies have implicated a paradoxical role of IFN- γ in controlling of auto-inflammation in Psoriasis. (18). Therefore, it may behave as a pro-inflammatory molecule to regulate immune response.

The current study showed hs-CRP levels were significantly increased in cases compared to controls. However, there was no statistically significant severity with respect to PASI score in cases. hs-CRP being both inflammatory and cardiovascular marker it was correlated with other inflammatory markers. Current study showed significant negative correlation with IFN- γ . The possible explanation for correlation between hs-CRP level and psoriasis severity is, keratinocytes in psoriasis lesion secretes interleukin-1 and tumor necrosis factor- α , which influences hepatocytes to release hs-CRP into the circulation, so mild psoriasis will have lower level of this marker than severe disease (19). A study by Coimbra *et al.*, also showed a positive correlation between hs-CRP and IL-6 (21). Their study showed an increase in hs CRP, including insulin resistance and adiposity which are common in patients with psoriasis, whilst in certain studies there was no correlation observed (20). Following antipsoriatic therapy, there was a decrease in hs-CRP and IL-6 levels which can further decrease the systemic inflammation and thus decrease cardiovascular comorbidities in these patients. A Few studies have shown that after treating for 12-week phototherapy CRP levels in patients decreased significantly.

Some authors have shown low levels of inflammatory cytokines (IL-22, IL-17, IL-23, IL-8, TNF- α , and VEGF vascular endothelial growth factor) after 12-week phototherapy (21). Following treatment there was a decrease in the level of CRP which is evidence of decreased burden of systemic inflammation and atherosclerosis in these subjects. Cytokines are small, biologically highly active proteins that regulate the growth, function, and differentiation of cells which progresses the immune response and inflammation.

Keratinocytes secrete varied cytokines and chemokines which either induce or decrease the immune response. However, there is no clarity in their mechanism of action in the pathogenesis of Psoriasis. Under the influence of either local or systemic stimulus these keratinocytes produce more cytokines (22). In psoriasis, there is a cutaneous and systemic over expression of various inflammatory cytokines and these cytokines could impact each other. Once the cutaneous inflammation is stimulated by the antigen, the macrophages, keratinocytes, Th1 cells, T17 cells, Th22 cells and BDCA-1-inflammatory dendritic cells, which will produce TNF- α . This TNF- α plays an important role in the inflammatory process in psoriasis (23). It stimulates the movement of Langerhans cells by lowering the level of e-cadherin and is involved in the NF-K β -mediated inflammation pathway, which contributes to cell survival, proliferation, and transcription of antiapoptotic factors (24).

Th1 cells produce IFN- γ which stimulates the transduction of signal and activation of Transcription (STAT) thus regulating expression of genes in psoriatic cutaneous tissue.

In addition, the transcription of IFN- γ and TNF- α can be regulated by IL-2 and IL-12. IL-2 plays a role in the differentiation, proliferation, and maturation of T lymphocytes. There is also abnormal differentiation of keratinocytes along with increased hyperplasia resulting in plaque formation, which is the key feature of IL-22 activity (8). In addition, TNF- α , IL-1 β , IL-6, and INF- γ were known to increase the production of C3 from the liver and probably from adipose tissue in psoriasis patients.

Thus TNF- α and IFN- γ produce constellation of inflammatory cytokines like IL-6, IL-8, IL-12 and IL-18 and provide a major link in cytokine network in psoriasis (25). These data confirm the hypothesis that psoriasis with altered immunologic pathway leads to systemic disease burden in these subjects. This impaired pathway is worsened with progression of the disease. Further it aggravates in the development of comorbidities associated such as cardiovascular risk factors including altered lipid levels, impaired glucose tolerance in these patients. As a result, this leads to increased risk for cardiovascular diseases in psoriasis with respect to their severity.

CONCLUSION

The present study concludes that the inflammatory markers are increased in psoriasis patients. These cytokines are considered as prognostic markers in psoriatic patients and there by providing appropriate therapeutic strategies for monitoring these patients in routine medical practice. And also, hs-CRP can be used to assess the cardiovascular risk in these patients.

In fact, hs-CRP test is simple blood test and is inexpensive which can be routinely used.

The new ELISA methods are useful with their high specificity and good standardizability against the high costs. These markers can be used as surrogate indicators to assess cardiovascular risk in psoriasis, who are most susceptible to develop co-morbidities. Thus, these simple biomarkers have an important role in the clinics for better treatment and prognosis and thereby reducing comorbidities in these patients.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

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